SUPPORTING INFORMATION FOR:

Title

Selective ligand behaviors provide new insights into agonist activation of nicotinic acetylcholine receptors

Author List

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Supplemental Table 1

	$(\alpha 4\ L9'A)_2(\beta 2)_3$					$(\alpha 4\ L9'A)_3(\beta 2)_2$				
Mutation	**EC_{50} (nM)**	**Hill**	**n**	**Fold Shift**	**I_{max} (μA)**	**EC_{50} (nM)**	**Hill**	**n**	**Fold Shift**	**I_{max} (μA)**
WT	1.1 ± 0.04	2.3 ± 0.2	21	–	3.3 – 35	0.32 ± 0.09	1.8 ± 0.8	21	–	0.95 – 13
β2Leu119	0.80 ± 0.04	1.9 ± 0.2	11	**0.7**	4.3 – 30	0.20 ± 0.03	1.9 ± 0.4	9	**0.6**	0.45 – 12
Lah	8.5 ± 0.2	1.4 ± 0.04	14	**10**	0.49 – 22	0.99 ± 0.03	1.8 ± 0.1	12	**5.0**	0.24 – 55
α4Thr155	0.65 ± 0.07	1.7 ± 0.3	13	**0.6**	1.9 – 6.4	0.16 ± 0.02	2.4 ± 0.4	14	**0.5**	0.79 – 5.8
Tah	3.7 ± 0.2	1.6 ± 0.1	12	**5.7**	0.52 – 8.6	1.8 ± 0.1	1.4 ± 0.1	14	**11**	0.43 – 12
α4Trp154	0.46 ± 0.03	1.8 ± 0.2	16	**0.42**	0.25 – 2.4	0.19 ± 0.02	1.7 ± 0.2	8	**0.59**	0.47 – 11
Trp-F1	0.78 ± 0.06	1.6 ± 0.2	18	**1.7**	0.089 – 0.93	0.38 ± 0.03	1.7 ± 0.2	8	**2**	1.1 – 8.1
Trp-Br	0.87 ± 0.06	1.8 ± 0.2	14	**1.9**	0.25 – 1.8	–	–	–	–	–
Trp-F2	1.4 ± 0.1	1.4 ± 0.1	20	**3.1**	0.049 – 0.74	0.70 ± 0.04	1.9 ± 0.2	8	**3.7**	11 – 34
Trp-CN	2.2 ± 0.1	1.7 ± 0.1	4	**4.8**	0.21 – 0.40	–	–	–	–	–
Trp-F3	3.5 ± 0.3	1.2 ± 0.1	11	**7.6**	0.27 – 1.3	4.6 ± 0.4	1.1 ± 0.1	8	**24**	0.30 – 1.1
Trp-F4	10.0 ± 0.7	1.1 ± 0.06	12	**22**	0.042 – 1.2	11 ± 1	0.89 ± 0.05	7	**58**	0.66 – 3.0

Agonist = Sazetidine-A

WT = wild type

Leu, Thr, Trp = wild type recovery control (nonsense suppression method with the wild type amino acid)

Lah = leucine-α-hydroxy

Tah = threonine-α-hydroxy

Trp-F1 = 5-flouro-tryptophan

Trp-Br = 5-bromo-tryptophan

Trp-F2 = 5,7-diflouro-tryptophan

Trp-CN = 5-cyano-tryptophan

Trp-F3 = 5,6,7-triflouro-tryptophan

Trp-F4 = 4,5,6,7-tetraflouro-tryptophan

Supplemental Table 2

Receptor	*Acetylcholine* EC_{50} (μM) [1]	Hill [1]	EC_{50} (μM) [2]	Hill [2]	% [1]	n	Imax (μA)	*Sazetidine-A* EC_{50} (nM)	Hill
$(\alpha4)_2(\beta2)_3$	0.78 ± 0.02	1.1 ± 0.02	–	–	–	16	0.15 – 2.4	1.9 ± 0.1	2.0 ± 0.2
$(\alpha4)_3(\beta2)_2$	2.7 ± 9	0.69 ± 0.54	100 ± 11	1.6 ± 0.3	17	8	9.8 – 27	**NR**	
$(\alpha4\ H114V)_3(\beta2)_2$	44 ± 80	1.1 ± 0.5	240 ± 50	2.6 ± 2	47	11	0.29 – 12	**NR**	
$(\alpha4\ Q122F)_3(\beta2)_2$	2.1 ± 3	0.64 ± 0.3	190 ± 20	1.8 ± 0.3	23	15	0.31 – 12	**NR**	***(too low curre***
$(\alpha4\ T124L)_3(\beta2)_2$	4.8 ± 14	0.92 ± 0.5	33 ± 9	1.8 ± 0.8	37	7	1.0 – 4.8	**NR**	
$(\alpha4\ H114V, Q122F)_3(\beta2)_2$	34 ± 60	1.0 ± 0.6	260 ± 40	4.0 ± 5	51	19	0.59 – 15	>2000	0.90 ± 0.1
$(\alpha4\ H114V, T124L)_3(\beta2)_2$	11 ± 20	1.1 ± 0.4	66 ± 22	2.0 ± 1	46	17	0.52 – 11	>2000	0.94 ± 0.1
$(\alpha4\ Q122F, T124L)_3(\beta2)_2$	0.74 ± 0.1	0.96 ± 0.1	–	–	–	10	0.067–0.22	**NR**	***(too low curre***
$(\alpha4\ H114V, Q122F, T124L)_3(\beta2)_2$	2.7 ± 0.2	0.81 ± 0.03	–	–	–	16	0.89 – 18	9 ± 1	2.3 ± 0.6

NR = No Response

Supplemental Table 3

$(\alpha 4\ L9'A)_2(\beta 2)_3$

	Acetylcholine					*Sazetidine-A*				
Mutation	**EC_{50} (μM)**	**Hill**	**n**	**Fold Shift**	**I_{max} (μA)**	**EC_{50} (nM)**	**Hill**	**n**	**Fold Shift**	**I_{max} (μA)**
WT	0.34 ± 0.01	1.2 ± 0.02	15	**–**	0.88 – 15	0.66 ± 0.04	2.1 ± 0.2	14	**–**	0.69 – 8.1
β2 V109H	1.3 ± 0.03	1.3 ± 0.03	15	**3.8**	1.5 – 13	7.7 ± 0.4	1.4 ± 0.1	12	**12**	1.2 – 5.2
β2 F117Q	1.1 ± 0.01	1.2 ± 0.01	14	**3.2**	0.53 – 10.	510 ± 30	1.4 ± 0.1	12	**770**	0.74 – 5.8
β2 L119T	20 ± 0.7	1.3 ± 0.05	14	**59**	1.7 – 12	180 ± 6	1.2 ± 0.04	14	**270**	0.87 – 12

WT = wild type

Supplemental Table 4

Receptor	EC_{50} (μM) [1]	Hill [1]	EC_{50} (μM) [2]	Hill [2]	% [1]	n	I_{max} (μA)
$(\alpha4)_2(\beta2)_3$	0.78 ± 0.02	1.1 ± 0.02	–	–	–	16	0.15 – 2.4
$(\alpha4)_3(\beta2)_2$	7.2 ± 23	0.7 ± 0.4	106 ± 13	1.7 ± 0.5	26	15	1.7 – 31
$(\alpha4)_2(\beta2)_3$ + 10 μM NS9283	0.48 ± 0.02	1.1 ± 0.05	–	–	–	12	0.095 – 7.2
$(\alpha4)_3(\beta2)_2$ + 10 μM NS9283	0.15 ± 0.004	1.07 ± 0.03	–	–	–	14	0.42 – 42
$(\alpha4)_2(\beta2\ F117Q)_3$	1.6 ± 0.1	1.6 ± 0.1	–	–	–	15	0.033 – 0.28
$(\alpha4)_3(\beta2\ F117Q)_2$	9 ± 15	0.9 ± 0.4	134 ± 22	1.6 ± 0.3	25	14	1.5 – 15
$(\alpha4)_2(\beta2\ V109H, F117Q, L119T)_3$	35 ± 1	1.5 ± 0.04	–	–	–	14	2.0 – 28
$(\alpha4)_3(\beta2\ V109H, F117Q, L119T)_2$	180 ± 10	1.6 ± 0.1	–	–	–	12	0.16 – 6.3
$(\alpha4\ H114V, Q122F, T124L)_2(\beta2)_3$	1.0 ± 0.03	1.1 ± 0.03	–	–	–	22	0.11 – 4.2
$(\alpha4\ H114V, Q122F, T124L)_3(\beta2)_2$	2.7 ± 0.2	0.81 ± 0.03	–	–	–	16	0.89 – 18

Agonist = Acetylcholine